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THE DEVELOPMENTAL ROLE OF SLEEP: A NEW HYPOTHESIS

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Introduction

It is well known that sleep prevails in late fetal life and early postnatal development [1-3]. To account for this basic feature, sleep is considered to play a role in brain development and maturation. This hypothesis was originally proposed by Roffwarg *et al.* [4] with regard to paradoxical sleep (PS) and was recently examined in experiments of PS deprivation (PSD) involving rats in their early postnatal life [5] or pregnant rats in late gestation [6, 7]. Under the latter conditions, DNA synthesis increased dramatically in fetal brain, thus providing a direct demonstration that PS exerts a significant influence on mammalian brain development. The programming of brain circuitry required for instinctual

behaviour has also been proposed to occur during PS [8]. In this connection, it is of considerable interest that, in the mature mammal, PS is known to be involved in memory processing, as shown by the selective PS increase observed following acquisition of complex tasks and by the drastic impairment in memory retrieval induced by post-training PSD [9, 10]. These findings indicate that PS plays a role in the remodelling of brain circuitry which occurs after learning. The latter role may not differ in any substantial way from the role played by PS in early brain development, except for the source of the information used in the modelling or remodelling of brain circuitry. This information is derived from internal sources during development while it originates from the environment during learning. The difference notwithstanding, it would appear that the developmental role of PS may well extend into the late part of the life cycle.

A major limitation of the above hypotheses is, however, their concern with PS only, thereby paying little or no attention to the other major type of sleep, that is synchronized sleep (SS). Independent hypotheses have been advanced with regard to the role of SS [11, 12], but again these proposals suffer from a similar drawback, in that they aim at explaining the function of SS, but pay little or no attention to the existence of PS.

We have been concerned with the lack of a unitary explanation of the role of sleep for some time [13, 14] and have recently outlined a conceptual framework which integrates the roles of SS and PS in the sleep-wakefulness cycle, suggesting furthermore some general guidelines for the experimental test of the hypothesis, most promisingly at the molecular level [15]. The hypothesis is called the sequential hypothesis, since it maintains that the operations performed by the sleeping brain follow a necessary temporal sequence, those requiring SS occurring first and preparing the stage for the later operations performed during PS. The nature of the operations remains to be defined, but the hypothesis postulates that they are related to the processing of the information

acquired during the previous period of wakefulness (W). Two important consequences derive from this formulation. In the first place, SS is considered to be involved in brain information processing and to play a major role in the initial modification of newly-acquired information which makes it amenable to the subsequent changes brought about by PS. The other consequence concerns the dependence of the operations performed by the sleeping brain on the nature of the previous waking experience. The sequence of events is actually believed to start during W and to continue sequentially during SS and eventually PS. In brief, if the new information acquired by the waking brain is called I_W, the sequential hypothesis may be stated as

$$I_W \rightarrow I_{SS} \rightarrow I_{PS}$$

in which the subscripts identify the vigilance states.

The sequential hypothesis initially suggested itself as an analogy to the processing of food [13]. Matter, energy and information are the main environmental inputs of the living organism, the first two being combined as food in the animal kingdom. During its intestinal journey, food is progressively degraded by mechanical and chemical processes before being absorbed. A key point is that these operations are carried out in sequential steps which take place in the main stations of the intestine. An additional point regards the activity of the gut (secretion and motility) which increases following the initial ingestion of food, but remains at a minimum in the interval between meals. The composition of the secretory juices is also known to change according to the nature of the food ingested. In accordance with the analogy, the processing of brain information was thought to take place in sequential steps occurring during SS and PS, respectively, provided new information was acquired by the brain during the previous period of W and according to the nature of that experience. More explicitly, we assumed that the sleeping brain initiates the sequential operations of information processing upon ac-

quisition of new information, while its activity remains low (even lower than during W) if the waking experience has not yielded novel inputs. As a result, it seems clear that to elucidate the operations performed by the sleeping brain, one should use animals exposed to an appropriate learning situation in the previous waking period. Following our analogy, this is the same elementary rule followed by physiologists studying digestion, in that they give food to their experimental animals, and most often food of a known composition. Yet, in practically all studies made on the sleeping brain, this consideration has been completely neglected [13, 14]. It may not be surprising, therefore, that some of these studies have yielded contrasting results. These discrepancies might be solved by taking into account the nature of the previous waking experience [14].

Two general approaches may be suggested for the experimental verification of the sequential hypothesis, a longitudinal approach and a transversal approach. The former method aims at deciphering the fate of the information gathered by the brain during W, provided sleep is allowed in the period immediately following W. In principle, this may be accomplished by any method capable of measuring I_W and its postulated sleep modifications I_{SS} and I_{PS}. Of the possible means to measure the correlates of brain acquisition (behavioural, physiological or neurochemical methods), the neurochemical approach appears perhaps the best suited to test the sequential hypothesis and to yield key information on the activities of the sleeping brain. Specific (macro)molecular changes associated with the acquisition of new information may be labelled by administering suitable radioactive precursors before the waking experience, and by comparing the quantitative and/or qualitative features of the radioactive brain product between trained and control animals. Likewise, the effect of sleep on I_W may be determined by letting animals enter a post-training rest period, and by relating the properties of the radioactive brain product analyzed at the end of the rest period

(e.g., concentration, regional and cellular distribution, molecular species) to the sleep parameters recorded during the same period. Additional experimental variables may be introduced in this general scheme by manipulating the post-training vigilance state with pharmacological or instrumental procedures.

On the other hand, the aim of the transversal approach is to measure the nature of the changes in brain activity which occur during sleep, following the acquisition of new information. The comparison is to be made with a similar period of sleep which follows the acquisition of little or no information. In this approach, radioactive precursors are to be given after the waking experience and before the rest period, to allow analysis of the biochemical behaviour of the brain during sleep. It is crucial that the waking experience of different animal groups be varied so as to amplify differences. The general scheme of the transversal approach is essentially the same as the one used by physiologists in their studies on the secretory and motor activity of a segment of the intestine after the ingestion of a certain type of food. The strict dependence of the metabolism of the sleeping brain on the nature of the previous waking experience is supported by literature data on the levels of brain energy metabolites [16] and on the rate of phosphorylation of brain glucose-6-phosphatase [17] in groups of rats subjected to different environmental experiences in the preceding period of W (for a review, see 14). It is likely that some of the controversial results on the biochemical and physiological behaviour of the sleeping brain may be reflecting the influence of different waking experiences, as already suggested [14]. More generally, it might be advisable to reconsider these studies under conditions in which the nature of the previous waking experience is controlled and explicitly varied among animal groups.

An interesting consequence of the sequential hypothesis regards two proposals on the role of SS, the anabolic hypothesis [11] and the energy-saving hypothesis [12]. According to the former hypothesis, sleep is considered an anabolic pe-

riod for the brain, while according to the latter hypothesis, the purpose of SS is to save energy. Direct experimental support for either hypothesis is not overwhelming and some controversial results have been reported [13, 14]. In the light of the sequential hypothesis, it seems possible that the experimental data yielding support or, alternatively, questioning the validity of either hypothesis may be brought into a more understandable framework, by considering that an increase or a decrease in a given brain metabolic reaction or in a given physiological parameter may be the result of the previous waking experience [14]. Presumably, a sleeping brain may switch into an "anabolic" mode or, alternatively, into an "energy-saving" mode, according to the nature of its previous waking experience. The former behaviour is more likely to result from the acquisition of new or stressful information which requires appropriate processing, while the latter behavior is to be expected from a brain whose previous waking experience has been quiet, unstressed and devoid of novel information. From this point of view, the anabolic hypothesis and the energy-saving hypothesis may be brought under the more general concepts of the sequential hypothesis.

Experimental verification of the sequential hypothesis

We have started to investigate the validity of the sequential hypothesis using the longitudinal approach. In our initial studies we have followed the post-training sleep behaviour of brain DNA synthesized during the training period. We made this rather unusual choice, at least with regard to brain information processing, since our evidence and that of the literature suggest that newly-synthesized brain DNA is involved in learning [18-25]. Our experimental design followed the general guidelines indicated above. Groups of adult female Wistar rats, injected intraventricularly with [3H]methylthymidine were subjected to a massive two-way active

avoidance training during a period of 4 hr, or were kept in their home cages. All rats were allowed to rest for an additional period of 3 hr before being killed. During the post-training period, sleep states were monitored by EEG procedures. The main result of the experiment may be summarized by the statement that in the group of rats exposed to the training procedure but unable to reach the learning criterion the specific activity of DNA in several brain regions was inversely related to the amount of post-training PS [26]. This relationship was lacking in the group of rats which mastered the task and in the control rats which remained in their home cages. We interpreted this data to indicate that brain DNA synthesized in association with the acquisition process may be degraded if the information to which it relates is unable to induce a meaningful adaptive response, as it occurs in the group of trained rats which were unable to learn. Since the lower specific activity of brain DNA was inversely related to PS, PS was supposed to play a major role in the shedding of irrelevant information, as also suggested by a recent hypothesis [27]. The inverse relationship with PS, that is, with a brain event mostly occurring in the last 2 hr of a 7.5 hr pulse, supported the view that the variation was more likely to be due to a degradative process rather than to a synthetic process. More direct evidence on this point is however lacking. On the whole, the data seems to support the sequential hypothesis of sleep function on the grounds that i) the fate of a newly-synthesized brain macromolecule is related to post-training PS; and ii) this relationship occurs only in the group of animals experiencing a certain environmental input during the pre-sleep period. It should be added that the relationship between post-training PS and a brain biochemical parameter in no way excludes the involvement of SS in the observed biochemical variations. Indeed, an alternative interpretation of the data suggests that an additional and unknown brain parameter may be held responsible for the DNA changes and the PS changes alike. It is not unreasonable to assume that some feature of SS might account

for or be related to this key brain variable. In a more recent experiment in which the vigilance state of non-learning rats was pharmacologically manipulated to yield subgroups of sleep-deprived and PS-deprived animals, the DNA specific activity in some brain regions was related to SS features, such as its amount and the average duration of SS episodes. These correlations appeared to depend on the nature of the animal's waking experience [28].

It seems that the sequential hypothesis may be able to open an entire new field of investigations dealing with the nature of brain information processing. In brief, in the longitudinal as well as in the transversal approach, one may vary the parameters of the waking experience, the parameters of post-training sleep and the biochemical or the physiological parameters under examination. By the appropriate combination of these three dimensions of experimental design, the nature of the operations performed by the sleeping brain should become progressively better understood.

The developmental role of sleep

As long as a new waking period follows a period of sleep, a new cycle of acquistion and processing of brain information is started, each cycle leaving the brain in a state in which newly-learned adaptive information has become better integrated into the previously existing information. Shedding of older, unused or non-useful information is likely to occur concurrently with the deposition of new and useful information [27, 29]. It seems reasonable to assume that, during a given sleep cycle, brain processing may also involve information already processed in previous cycles. This is required by the idea that brain is structured as an integrated whole rather than as a collection of added parts. In principle, the study of the processing of information acquired some time before a given sleep period could follow the same general guidelines already

mentioned with regard to the longitudinal approach, that is, it could be based on the examination of the long-term, rather than the short-term fate of a given biochemical (or other) correlate of the acquisition step. Questions as to the lifetime of that correlate and to its possible replacement by variables of a different nature become of relevance in the attempt to assess the time interval in which such studies might achieve feasibility. Obviously, these investigations should be postponed until the "within cycle" studies will yield some clarifying insight.

Perhaps the most intriguing question raised by these thoughts concerns the initial stages of development of brain circuitry, during which the directing information is largely derived from internal sources. Is the initial modelling of brain circuitry controlled by different rules from those regulating the remodelling which occurs in later stages on the basis of environmental sources of information? Can we expect to learn something about the nature of the modelling process by the understanding of the remodelling steps? It would be hazardous to take a definite stand now, when we know so little of either mechanism. It seems nonetheless reasonable to assume, on a first approximation, that the nature of the initial modelling process may not be entirely different from that of the remodelling process. If this view is correct, the sequential hypothesis of sleep function might bring us closer to an understanding of the role of sleep in the initial periods of brain development. In planning experiments along this line, however, the drastic differences in sleep structure prevailing in the early developmental stages become soon evident. Indeed, only a more primitive form of PS occurs in these stages, while W and SS differentiate only at later stages [3-5, 30]. Since the source of the information directing the modelling of brain circuitry is largely intrinsic to the organism in the early developmental stages, it is tempting to speculate that the nature of this information (its operational form) may not be dissimilar from that originating from the environment, but eventually modified by SS in more mature brains. It would

seem that the need for an additional processing step arises only when the directing information becomes progressively more dependent on environmental sources, that is, when W starts to differentiate. This is presumably a consequence of the different form in which newly-acquired information is stored in brain during W, in comparison with innate information. In this light, the modificatoins brought about by SS may be serving the purpose of accomplishing this transformation which would then allow the further operations occurring during PS. If this reasoning is correct, the remodelling which takes place in mature brains as a result of experience would become more credibly similar, at least in its last stage, to the type of processing by which innate information directs the initial formation of brain circuits during earlier developmental stages.

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